

Listing of Claims

What is claimed is:

1. (Currently amended) A nucleic acid sequence comprising:

Px-Sx-Bn-(ZR)-Hir(AsmR)-protein(Y)-T

where

Px is a promoter sequence;

Sx is a nucleic acid encoding a signal sequence or leader sequence;

Bn is 1-15 codons, when n is an integer from 1 to 15, or a chemical bond, when $\underline{n=0}$ $\underline{n=O}$;

Z is a codon for lysine or arginine;

R is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence coding ~~for~~ hirudin or lepirudin;

~~Asm~~ Asm is a chemical bond, when $\underline{m=0}$ $\underline{m=O}$, or 1-10 codons, when m is an integer from 1 to 10;

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; and

T is an untranslated expression-enhancing nucleic acid sequence.

Claims 2 to 6 (Canceled)

7. (Original) A multicopy vector comprising the nucleic acid of claim 1.

8. (Original) A plasmid comprising the nucleic acid of claim 1.

9. (Original) A host cell comprising the nucleic acid of claim 1, as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally.

10. (Original) The host cell of claim 9, wherein the host cell is a yeast.

11. (Original) The host cell of claim 10, wherein the yeast is selected from *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, *Hansenula polymorpha*, and *Pichia pastoris*.

12. (Original) A host cell comprising the multicopy vector of claim 7.

13. (Original) A host cell comprising the plasmid of claim 8.

14. (Currently amended) A process of fermentative production of a hirudin-mini-proinsulin fusion protein comprising expressing the nucleic acid of the host cell of claim 9 to form the fusion protein in a fermentation supernatant of a cell culture.

Claims 15 to 20 (Canceled)

21. (Previously presented) The process of claim 14 further comprising isolating said fusion protein from said fermentation supernatant.

22. (Previously presented) The process of Claim 21 wherein said isolating the fusion protein comprises adjusting the pH of said fermentation supernatant to about 2.5 to 3.5 to precipitate non-desired proteins and form a precipitation supernatant and isolating the fusion protein from said precipitation supernatant.

23. (Previously presented) The process of claim 14 further comprising:
(A) separating the fermentation supernatant from the host cell; (B) culturing the host cell in fresh medium; (C) separating the resulting supernatant from the host cell; (D) repeating (B) and (C) several times; and (E) isolating the fusion protein from the aforementioned supernatants by adjusting the pH of said supernatants to about 2.5 to 3.5 to precipitate non-desired proteins and form a precipitation supernatant and isolating the fusion protein from said precipitation supernatant.

24. (Previously presented) The process of claim 21, wherein the isolating the fusion protein comprises precipitating the fusion protein from the fermentation supernatant, and further comprising releasing the protein encoded by protein(Y) from the fusion protein and concentrating said protein encoded by protein(Y) by microfiltration, hydrophobic interaction chromatography, ion exchange chromatography, or a combination of such procedures.

25. (Currently amended) A process of fermentative production of a hirudin-mini-proinsulin fusion protein, comprising expressing the nucleic acid of the host cell of claim 12 to form the fusion protein in a supernatant of a cell culture.

26. (Previously presented) The process of claim 25 further comprising isolating the fusion protein from the supernatant of the cell culture.

27. (Currently amended) A process of fermentative production of a hirudin-mini-proinsulin fusion protein, comprising expressing the nucleic acid of the host cell of claim 13 to form the fusion protein in a supernatant of a cell culture.

28. (Previously presented) The process of claim 27 further comprising isolating the fusion protein from the supernatant of the cell culture.

29. (Canceled)

30. (Currently amended) ~~A~~ The The process of claim 21 further comprising releasing mini-proinsulin by treating said fusion protein with trypsin and carboxypeptidase B.

31. (Currently amended) ~~A~~ The The nucleic acid sequence of claim 1 in which (AsmR), taken together, is an arginine codon.

32. (Currently amended) ~~A~~ The The nucleic acid sequence of claim 1 ~~2~~ in which (AsmR), taken together, encodes SEQ ID NO: 12 (Gly-Asn-Ser-Ala-Arg).

33. (Currently amended) ~~A~~ The nucleic acid sequence of claim 1 ~~2~~ in which Px is a yeast ADH2 promoter, Sx is an a factor leader sequence, and Hir encodes hirudin or lepirudin, (AsmR), taken together, is either an arginine codon or encodes SEQ ID NO:12 (Gly-Asn-Ser-Ala-Arg)}.

34. (Currently amended) ~~A~~ The nucleic acid sequence of Claim 33 wherein protein(Y) is a nucleic acid sequence encoding mini-proinsulin.

35. (Currently amended) ~~A~~ The nucleic acid of Claim 34 wherein Hir is a nucleic acid sequence encoding lepirudin which has been prepared recombinantly.